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The effect of carbon dioxide on the twinkling artifact in ultrasound imaging of kidney stones: A pilot study

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Abstract

Bone demineralization, dehydration, and stasis put astronauts at an increased risk of forming kidney stones in space. The color-Doppler ultrasound “twinkling artifact”, which highlights kidney stones with color, can make stones readily detectable with ultrasound; however our previous results suggest twinkling is caused by microbubbles on the stone surface which could be affected by the elevated levels of carbon dioxide found on space vehicles. Four pigs were implanted with kidney stones and imaged with ultrasound while the anesthetic carrier gas oscillated between oxygen and air containing 0.8% carbon dioxide. Upon exposing pigs to 0.8% carbon dioxide, twinkling was significantly reduced after 9–25 minutes and recovered when the carrier gas returned to oxygen. These trends repeated when pigs were again exposed to 0.8% carbon dioxide followed by oxygen. The reduction of twinkling from exposure to elevated carbon dioxide may make kidney stone detection with twinkling difficult in current space vehicles.

Keywords

ultrasound; kidney stones; Doppler; space; bubbles; twinkling artifact; carbon dioxide

Introduction

Astronauts are at an increased risk of forming kidney stones because of bone demineralization, dehydration, stasis, and alkalinization of urine that occur in spaceflight (Jones et al. 2008; Sibonga et al. 2008). While generally innocuous in the kidney, a stone

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often causes debilitating pain as it passes, and even worse, may become obstructive, which could lead to sepsis, urinary tract infection, renal failure, or even death (Glowacki et al. 1992; Pearle et al. 2005; Scales et al. 2012). Current ground-based technologies to detect kidney stones, such as plain-film x-ray or computed tomography, are unsuitable for flight because of the equipment size and/or exposure to ionizing radiation. B-mode ultrasound can also be used to detect kidney stones; however the sensitivity of the technique is highly dependent upon the skills of the operator and has been reported as low as 30% (Fowler et al. 2002; Ulusan et al. 2007). The color-Doppler ultrasound "twinkling artifact," (TA) which highlights hard objects such as kidney stones with rapidly changing color, has been shown to improve the sensitivity of ultrasound for stone detection (Dillman et al. 2011; Lee et al. 2001; Mitterberger et al. 2009; Rahmouni et al. 1996; Winkel et al. 2012); however the inconsistent appearance of the TA has limited its use in the clinic. Recently, Lu et al. proposed that stable crevice microbubbles on the kidney stone surface cause twinkling (Lu et al. 2013), and bubbles are expected to be very sensitive to aspects of space travel including changes in ambient pressure and the composition of vehicle air. This paper reports the influence of one aspect of space travel, namely exposure to elevated levels of carbon dioxide, on twinkling in an *in vivo* porcine pilot study.

Twinkling is an artifact that was first observed by Rahmouni et al. in 1990 (Rahmouni et al. 1996). Several explanations have been proposed to explain twinkling including: the interaction of the acoustic field with the stone; an imperfection in the ultrasound machine; and more recently, stable crevice microbubbles on the kidney stone surface (Chelfouh et al. 1998; Kamaya and Rubin 2003; Lu et al. 2013). The primary evidence for stable crevice bubbles on the stone surface is that twinkling is suppressed by hydraulic overpressure and returns when the overpressure is released (Lu et al. 2013). Overpressure is known to shrink bubbles and drive them into solution. Bubbles in crevices are stabilized against dissolution from overpressure because the meniscus turns inward and surface tension acts to stabilize, not dissolve the bubbles (Apfel and Holland 1991). Since twinkling was first suppressed by overpressure then observed to return when the overpressure was released, this supports the hypothesis that crevice microbubbles exist on the kidney stone surface; none of the other proposed mechanisms are affected by static overpressure. Furthermore, when a kidney stone was submerged in ethanol, a wetting agent with a surface tension much lower than water, twinkling was suppressed as the ethanol displaced the bubbles from the crevices (Lu et al. 2013). While these results show strong evidence for twinkling being caused by crevice microbubbles on the kidney stone, the hypothesis is still under debate as the bubbles have not been directly observed (Tanabe et al. 2014). Nevertheless, specific conditions that occur in spaceflight are likely to perturb these bubbles and therefore influence the appearance of twinkling on kidney stones.

On the International Space Station, reported carbon dioxide levels have generally ranged from 2.3 to 5.3 mm Hg (10–20 times the concentration on Earth equivalent to 0.4–0.8% CO_2), with significant spatial and temporal variations (Alexander et al. 2012; James et al. 2011; Law et al. 2010). Local pockets of carbon dioxide form due to reduced air convection in microgravity; according to a computational fluid dynamics model, without sufficient ventilation these pockets of carbon dioxide can reach 9 mm Hg in as little as 10 minutes when the astronaut is sleeping (Alexander et al. 2012). The elevated concentrations of

carbon dioxide have been shown to correlate with the reported incidence of headaches and have also been thought to contribute to increased intracranial pressure (Alexander et al. 2012; Law et al. 2010; Law et al. 2014). Because of the link between symptoms and carbon dioxide levels, flight surgeons have lowered their action threshold to 5 mm Hg from the flight rule permissible exposure limit of 7.6 mm Hg (Law et al. 2010). Carbon dioxide is almost thirty times more soluble in blood than oxygen (O'Brien and Parker 1922) and long-term exposure to elevated carbon dioxide levels has been suggested to increase the risk of kidney stone formation because of the increased alkalinity of the urine (Brandis n.d.; Tomoda et al. 1995). The variation in gas concentrations in the tissue and urine as well as the physiological adaptations from exposure to elevated levels of carbon dioxide are likely to influence the formation and appearance of all bubbles in the human body, including those that may be present on the surface of kidney stones.

The goal of this paper is to present experimental evidence from a pilot study that exposure to elevated levels of carbon dioxide reduces the magnitude and persistence of the color-Doppler ultrasound twinkling artifact in an *in vivo* porcine model. Pigs were exposed to an anesthetic carrier gas that oscillated between oxygen and elevated carbon dioxide at 8000 ppm in air (20x the concentration of carbon dioxide on Earth, the upper end of the reported ambient carbon dioxide levels found on the International Space Station) while twinkling was monitored qualitatively during the course of the experiment and quantitatively from the saved in-phase quadrature (I/Q) data.

Materials and Methods

All animal procedures have been approved by the University of Washington Institutional Animal Care and Use Committee. Five farm-bred pigs were injected intramuscularly with Telazol premedication before being intubated and maintained at a surgical plane with isoflurane in oxygen. The animals were instrumented to monitor heart rate, blood oxygenation, temperature (Ultraview SL, Spacelabs Healthcare, Snoqualmie, WA, USA) respiration rate and the partial pressure of exhaled carbon dioxide (CO_2SMO, Novametrix Medical Systems, Wallingford, CT, USA). The abdomen and torso of the animal was carefully shaved and de-pilated with Nair® to improve ultrasound coupling. Then, *ex vivo* human calcium oxalate monohydrate kidney stones (approximately 4-mm diameter) that had been stored in water for at least a week and known to display the TA *in vitro* were implanted into the animals via retrograde ureteroscopy. Care was taken to place the two stones in separate calyces of the kidney. At the end of the stone implantation, a catheter was left in the bladder for urine collection. Using a Doppler-guided ultrasound needle, a catheter was placed in the femoral artery for blood collection, and baseline blood and urine samples were collected. The kidney was imaged with a Verasonics® research ultrasound system (Redmond, WA, USA) and Philips/ATL P4-2 (Bothell, WA, USA) transducer to locate the stones by a sonographer. The stone with the best visualization of ultrasound twinkling was chosen for the study, after which the transducer was clamped in place for the duration of the study with only minor adjustments to position. A baseline twinkling was collected for at least 15 minutes before the anesthetic gas carrier was changed in four of the pigs from oxygen to elevated carbon dioxide at 8000 ppm in compressed air; the fifth pig was maintained on oxygen for more than one hour to serve as a sham. The four treated pigs

continued to breathe the increased carbon dioxide levels for two hours or until the oxygen saturation levels dropped below 45%, at which point the anesthetic gas carrier was switched back to oxygen. After about thirty minutes on oxygen, the pigs were again exposed to the elevated carbon dioxide levels until the oxygen saturation levels dropped below 45% at which time the pigs were once again returned to oxygen. Blood and urine samples were collected every thirty minutes and immediately before changing the anesthetic carrier gas.

Twinkling was monitored in real-time during the oxygen and elevated carbon dioxide exposures and a screen capture video of the experiment was recorded. In addition, the in24 phase quadrature (I/Q) Doppler data were saved at one frame per second continuously over the course of the entire experiment, a rate chosen for its minimal impact on the frame rate of the displayed image. A standard color-Doppler ultrasound programming sequence was used for imaging, with 9 Doppler ensembles emitted consisting of 3 cycles at 3000 Hz, interleaved with the standard B-mode ultrasound imaging; the frequency of the ultrasound transducer was 2.5 MHz. The power of the Doppler pulses and the Doppler power threshold, or the Doppler power at which color is displayed on the image, were adjusted to minimize saturation and splay artifact (a banding effect related to the point-spread function of plane wave imaging). In two of the pigs, stones were placed such that both stones were clearly visible in the ultrasound image throughout the course of the study, so twinkling was analyzed for both stones.

After the study, the I/Q data was imported into MATLAB (MATLAB 7.3, MathWorks, Natick, MA, USA) for quantification of twinkling. As in standard color-Doppler processing, the returns from the first two ensembles were omitted to avoid possible unrepeatable tissue reverberation. I/Q data were then filtered with a quadratic regression filter, a region-of-interest was selected that encompassed the stone, and the magnitude of the Doppler signal at each pixel was summed over the region-of-interest. The calculated value is the twinkle power or the Doppler magnitude summed over a region-of-interest of fixed size containing the stone. This measurement encompasses the brightness of individual pixels as well as the number of pixels with the twinkle signal and can be averaged over time to detect persistence. Outliers, which were defined as frames with the twinkle power exceeding 1.5 times the interquartile range, were removed from the analysis as these were frames showing noise associated with probe manipulation as verified by viewing the individual frames. The magnitude of the Doppler signal was also calculated over two averaged background regions-of-interest (size-matched to the stone region-of-interest), selected away from the stone and other strong signals such as blood vessels. This background signal was subtracted frame-wise from the twinkle power calculated from kidney stone to eliminate possible variations in twinkling from changes in the ultrasound propagation (e.g., changes in transducer coupling, changes in the tissue gas concentrations, etc.).

For each pig, the twinkle power of the stone minus the background signal was plotted in one-second increments over the course of the study. As the exposure time for the elevated carbon dioxide levels depended upon the biological response of each pig, it was necessary to develop a method to compare twinkling between pigs and anesthetic carrier gas composition. Therefore, the mean and standard deviation for eight-minute intervals at the middle and end of each gas exposure was calculated and used for the statistical analysis. Using a paired t-test

and considering all stones as independent, p-values were calculated to determine whether the summed twinkling power over the region of interest differed when pigs were exposed to the different breathing gases. P-values less than 0.05 were considered significant.

Every 30 minutes or immediately before a change in the anesthetic carrier-gas composition, blood and urine samples were collected. Three milliliter blood samples were collected in heparin-lined syringes and blood analysis was immediately performed by the University of Washington Medical Center Clinical Lab (Seattle, WA, USA). Results included pH, carbon dioxide partial pressure, oxygen partial pressure, bicarbonate levels, and oxygen saturation levels. Approximately 8-mL urine was also collected and analyzed by a veterinary laboratory (Phoenix Central Laboratories, Mukilteo, WA, USA) blinded to the exposure condition for pH, specific gravity, protein, color, bacteria, crystals, etc. In addition, a carbon dioxide analysis (calculated from measured bicarbonate levels) was run on the urine samples.

Results

In all four pigs, twinkling was reduced or eliminated upon exposure to 8000 ppm carbon dioxide and increased or returned when pigs were exposed to pure oxygen as shown in fig. 1. These trends repeated when pigs were again exposed to 8000 ppm carbon dioxide followed by pure oxygen. Fig. 2 shows the quantified reduction in twinkling from exposure to carbon dioxide (shaded) and increase in twinkling upon exposure to oxygen (white). There is an observable phase lag in the decrease or increase in the signal as the animal breathes a new gas. Nevertheless, the signal rises while the pig breathes oxygen and falls when the pig breathes carbon dioxide. Statistical analysis indicates the twinkle power at the end of the first carbon dioxide was significantly different than the baseline or initial twinkle power ($p=0.0165$), and the twinkle power at the end of the first carbon dioxide exposure was significantly different than the twinkle power at the end of the first return to oxygen ($p=0.0086$). Twinkling at the end of the first return to oxygen was also significantly different than twinkling at the end of the second return to oxygen ($p=0.013$); no other comparisons were statistically significant.

In general, the time course of the decay in twinkling for each animal exposed to carbon dioxide tracked with the changes in blood oxygenation levels, meaning the animals are more sensitive to the carbon dioxide, as evident by a more rapid decrease in the oxygen saturation levels, showed a more rapid decay in twinkling. Three of the four pigs were very sensitive to the carbon dioxide exposure with animal 3 so sensitive it was necessary to terminate the study at the end of the first carbon dioxide exposure. In the pigs more sensitive to the carbon dioxide, the twinkle power was reduced in as little as 9–17 minutes after the exposure to the 0.8% carbon dioxide and was later eliminated. An example of the time course of twinkling for animal 1 is shown in fig. 3. In the plot, it is evident that twinkling dropped sharply upon exposure to carbon dioxide (shaded) and was virtually eliminated after 25 minutes. Animal 2 was not as sensitive to the changes in carbon dioxide, as evidenced by the real-time monitoring of the blood oxygenation levels. While twinkling was eventually reduced, this animal was maintained on the carbon dioxide for the full two hours in the first carbon dioxide exposure with blood oxygenation levels never dropping below 50%. All of the pigs

were more sensitive to the carbon dioxide for the second exposure, with the reduction in oxygen saturation levels and corresponding twinkle power dropping much more rapidly than in the first carbon dioxide exposure.

To ensure that the trends observed were not an artifact from the experimental protocol, one pig was a sham exposure where kidney stones were implanted and imaged for more than one hour with no changes in the anesthetic carrier gas composition from the standard pure oxygen; the twinkling amplitude did not appreciably change over the hour.

Throughout the course of the study, a reduction in the brightness of the B-mode image was also qualitatively observed in pigs exposed to the elevated carbon dioxide. While the I/Q B-mode data was not recorded for this study, an analysis of the screen-capture video revealed a significant reduction in image intensity of 5.7±1.9% ($p=0.0066$) over the course of the study for those pigs that were twice exposed to the elevated carbon dioxide levels; animal 3 showed less than a 1% change in the image intensity before removal from the study at the end of the first elevated carbon dioxide exposure. On the other hand, the sham pig showed an increase in the image intensity of 7.4% over the course of the experiment, the opposite of what was observed in the pigs exposed twice to the elevated levels of carbon dioxide. Changes in B-mode image brightness did not track with changes in anesthetic carrier gas, nor did the image brightness decrease gradually over time.

Blood and urine were collected for analysis approximately every 30 minutes or immediately before a change in the breathing gas composition. Table 1 separates out all analyzed parameters into those that were relatively stable (with only minor variations) and those that showed variations. All of the blood parameters showed variations, with only the partial pressure of oxygen and oxygen saturation parameters showing consistent increases upon exposure to oxygen and decreases upon exposure to carbon dioxide. Other parameters measured in the blood such as the bicarbonate, base excess, pH, and the partial pressure of carbon dioxide displayed inconsistent increases and decreases between measurements that did not track overall or with the anesthetic carrier gas composition. Fig. 4 includes plots of the partial pressures of oxygen and carbon dioxide in the blood. Fig. 4.b., the plot of the partial pressure of oxygen, clearly illustrates the decrease upon exposure to carbon dioxide and the increase upon exposure to oxygen. On the other hand, fig. 4.a., the plot of the partial pressure of carbon dioxide, at times increases or decreases with no relation to the exposure to oxygen or carbon dioxide. In the urine (table 1), the stable parameters listed showed little to no variation between samples in a single pig, with many of the results negative for all samples. The parameters that showed variations in the urine had inconsistent increases and decreases between measurements within the same pig, which did not track with the anesthetic carrier gas composition or overall.

Discussion

For the first time, this pilot study demonstrates that diagnostic ultrasound in the form of the color-Doppler ultrasound twinkling artifact is significantly influenced by acute exposure to elevated levels of inhaled carbon dioxide, which has strong implications for detecting kidney stones in flight. All four pigs exposed to 0.8% carbon dioxide showed a significant reduction

or elimination of twinkling in as little as 9 minutes after exposure to the increased levels of carbon dioxide; a reduction in the brightness of the B-mode image was also observed over the course of the study. This rapid decay in twinkling (and return of twinkling from exposure to oxygen) tracks well with the changes in the blood oxygenation levels, suggesting that blood oxygenation may be more important than blood carbonation levels or urine composition (at least for those parameters measured in this study) in determining the mechanism by which twinkling is influenced by inhalation of elevated levels of carbon dioxide.

As the flight environment includes long-term, rather than acute, exposure to the elevated levels of carbon dioxide, it is important to develop an understanding of the mechanism by which twinkling is reduced. While the compensatory biological mechanisms resulting from exposure to elevated levels of carbon dioxide are generally understood (Brandis n.d.), it is not clear how exposure to elevated levels of carbon dioxide and the resulting gas exchanges between the blood, urine, and tissues reduce twinkling. In acutely hypercapnic rats, it has been shown that the partial pressure of carbon dioxide is increased in the urine by the end of the first hour (first data point in the study) compared to the normocapnic controls (Batlle et al. 1985). This result is supported by cavitation literature, where it is well-known that the high solubility of carbon dioxide increases the number of gas molecules in the solution (Carstensen et al. 1993). Yet the observation that twinkling tracks with changes in blood oxygenation means twinkling could also be related to changes in oxygen or even nitrogen partial pressures. In decompression sickness literature, an “oxygen window”, exists where cellular metabolism of oxygen reduces the partial pressure of oxygen in the tissue compared to the blood (Blatteau et al. 2006). Similarly, the renal vascular structure makes the partial pressure of oxygen particularly low in the urine and renal medulla (Hwang et al. 1998; Leonhardt and Landes 1965). Thus, the reduction in oxygen from 100% to approximately 20% in the air balance of the carbon dioxide mixture could be reducing the amount of dissolved oxygen in the urine thereby reducing twinkling. More research is needed including measurements of the partial pressures of oxygen, nitrogen and carbon dioxide in the urine and tissues to determine the speed at which gas exchange occurs between the kidney and urine.

There were several limitations to the study design that could affect whether a similar reduction in twinkling is observed in flight. First of all, as pigs do not spontaneously form kidney stones, it was necessary to implant *ex vivo* human kidney stones. While the stones were submerged in water for at least one week before the study, they were exposed to air both when originally removed from the human subject and upon implantation in pigs. These exposures to air could change the quantity and types of gas on the stone surface from the *in situ* condition thereby influencing the observed reduction in twinkling from the elevated carbon dioxide exposures. Another limitation to this study is that twinkling was compared between elevated carbon dioxide in air and pure oxygen, rather than ambient air. The effects of exposure to pure oxygen on twinkling is unknown, and the change in the oxygen or even nitrogen concentrations, rather than the elevated carbon dioxide levels, could be the mechanism by which twinkling is reduced. A final limitation to this study is the inability to measure the partial pressures of carbon dioxide and oxygen in the urine, which could help

determine the mechanism by which twinkling is reduced and help us postulate whether a similar effect on kidney stone twinkling could occur in flight.

Conclusions

The results from this study continue to support the hypothesis that the twinkling artifact is caused by bubbles. In all animals, we observed a significant reduction in kidney stone twinkling from exposure to 0.8% carbon dioxide in air and observed the recovery of twinkling from exposure to oxygen; however more research is needed to determine the exact mechanism by which twinkling is reduced. Nevertheless, this pilot study showed for the first time that diagnostic ultrasound is influenced by exposure to elevated levels of carbon dioxide, which has important implications for spaceflight. Besides influencing kidney stone detection, exposure to elevated carbon dioxide levels should be considered when developing ultrasound-based therapeutics for spaceflight and when evaluating the risk of decompression sickness before extra-vehicular activities. Furthermore, the increase of twinkling from exposure to oxygen suggests that inhalation of oxygen may serve as an effective countermeasure to reduce the influence of carbon dioxide for all bubble-based technologies. Immediate future work includes testing the influence of different carbon dioxide concentrations on twinkling as well as potential countermeasures to restore twinkling in the porcine model. Other future work that is currently in the planning stages includes a human subject trial to explore the effects of hypercapnia on kidney stone detection with twinkling.

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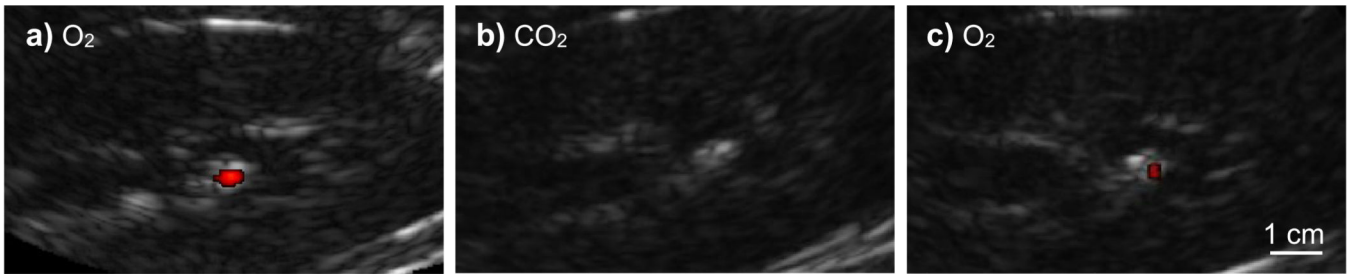

Fig. 1.

Representative frames of a video showing the general trends in twinkling from a) the initial exposure to oxygen (O_2), followed by the exposures to b) carbon dioxide (CO_2) then c) oxygen (O_2). a) Image showing a representative image showing the twinkling signal in red on the black and white image background. This was taken shortly after stone implantation and strong color isolated on the stone is seen. b) Image showing the stone 30 minutes after exposure to carbon dioxide with complete elimination of twinkling. c) Image showing twinkling on the stone 30 minutes after the pig was returned to oxygen. Twinkling (in red) has returned but at a level below what was originally observed in a). Figure is in color online.

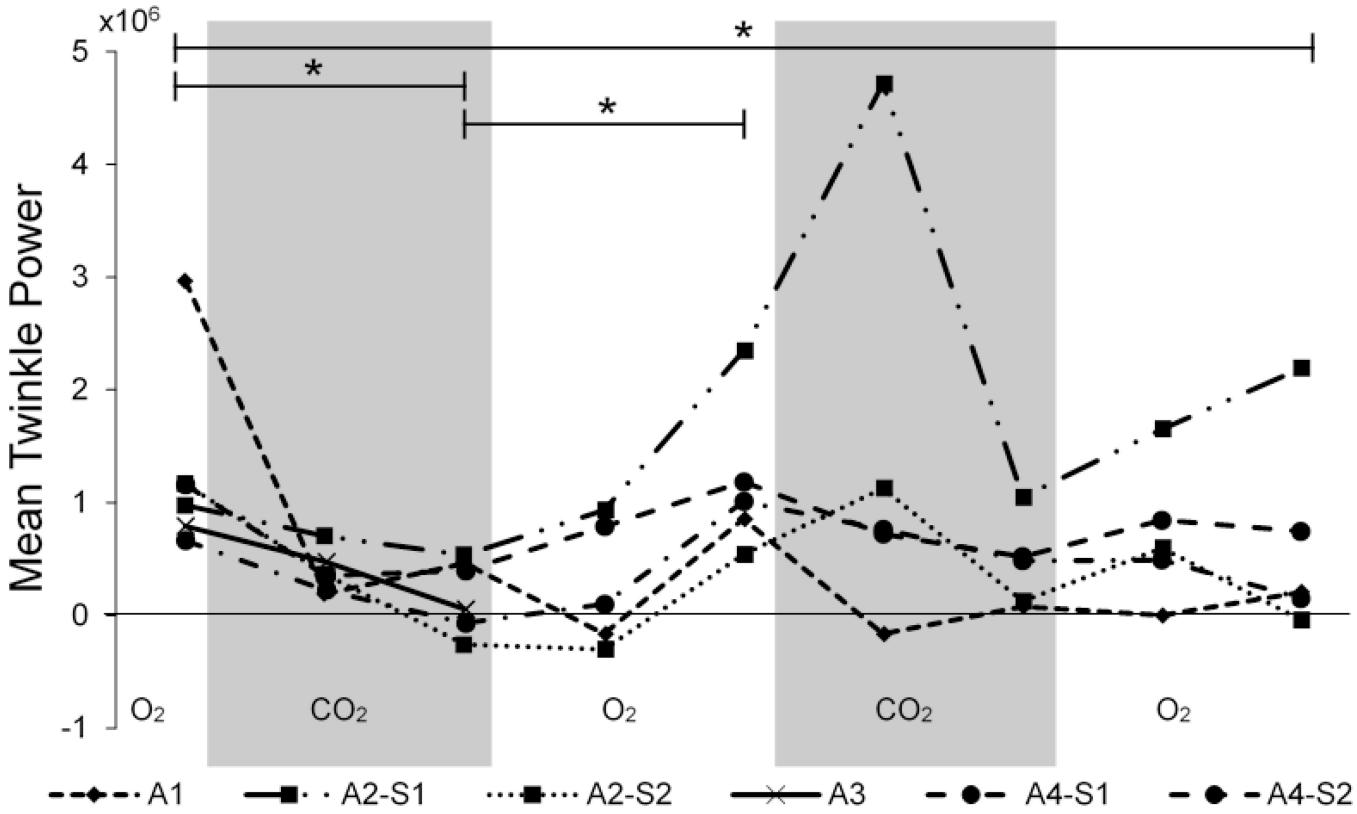

Fig. 2.

Summary plot showing the mean twinkle power calculated for 8-minute intervals at the middle and end of each carbon dioxide or oxygen exposure for all four animals. In general, twinkling is reduced at the end of each carbon dioxide exposure and is increased at the end of each oxygen exposure. In the middle of particularly the second carbon dioxide exposure, there are a few outliers where twinkling increased; however in these cases twinkling still ended at a lower level than was observed before the gas change. Three comparisons between values at the end of each gas exposure were significant as indicated by the lines and asterisks. As noted previously, animals 2 and 4 (A2 and A4) had two stones in the ultrasound imaging window throughout the course of the study and both are tracked here.

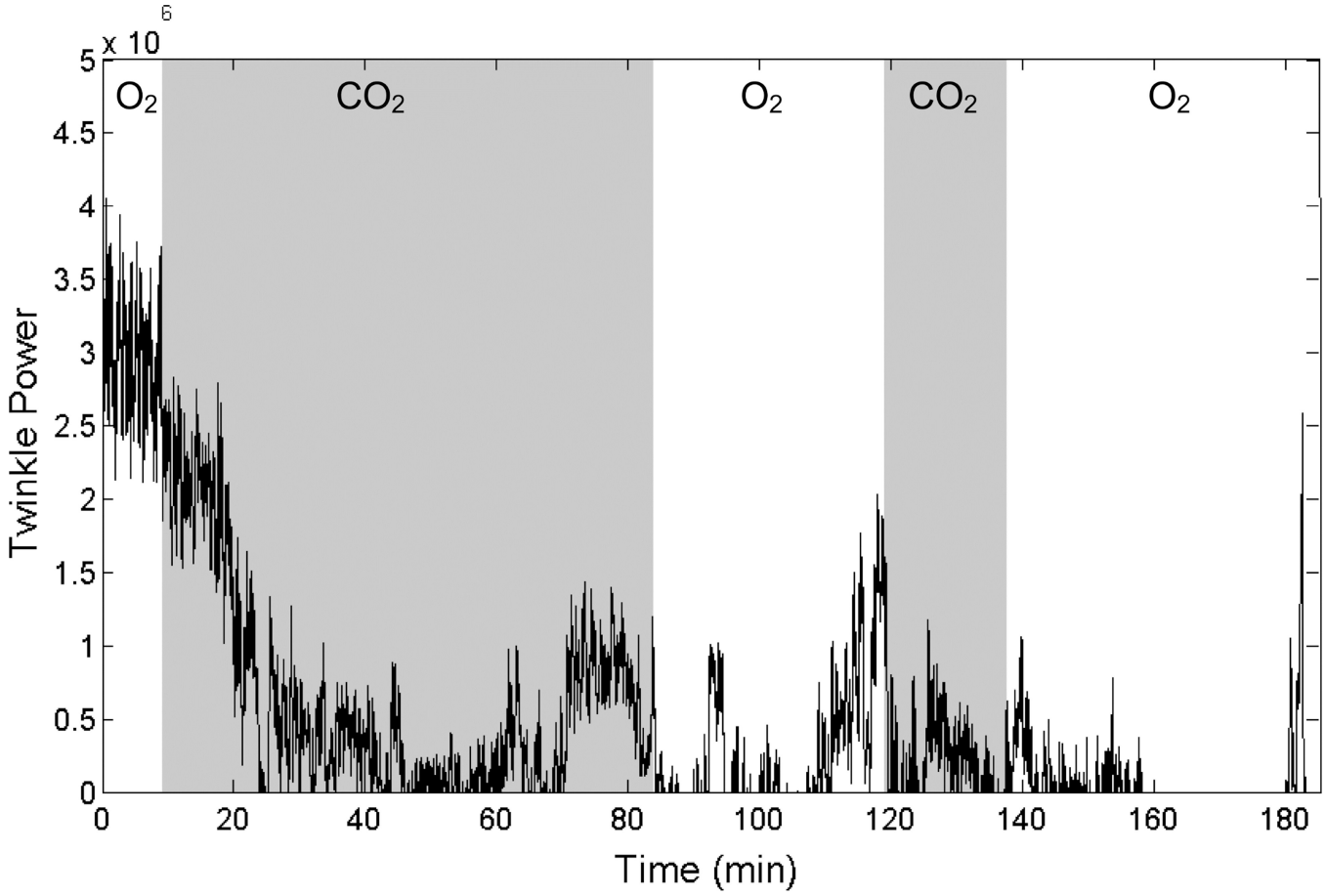

Fig. 3.

Plot showing the averaged twinkle power over time, an example from animal 1 showing the time course by which the magnitude of twinkling dropped from exposure to 0.8% carbon dioxide. Near the end of the first carbon dioxide exposure, twinkling rose slightly which is likely due to minor probe repositioning to ensure that twinkling was truly reduced. This slight increase in twinkling at the end of the first carbon dioxide exposure dropped sharply just before the pig was switched back to oxygen. In the time period 85–120 minutes, or the first return to oxygen, it took approximately 20 minutes for twinkling to begin to increase. When the pig was once again exposed to carbon dioxide, twinkling dropped abruptly and didn't start to return until about 40 minutes later on oxygen. Note: the data from 160–180 minutes failed to save, so the twinkle power is not 0 as it appears.

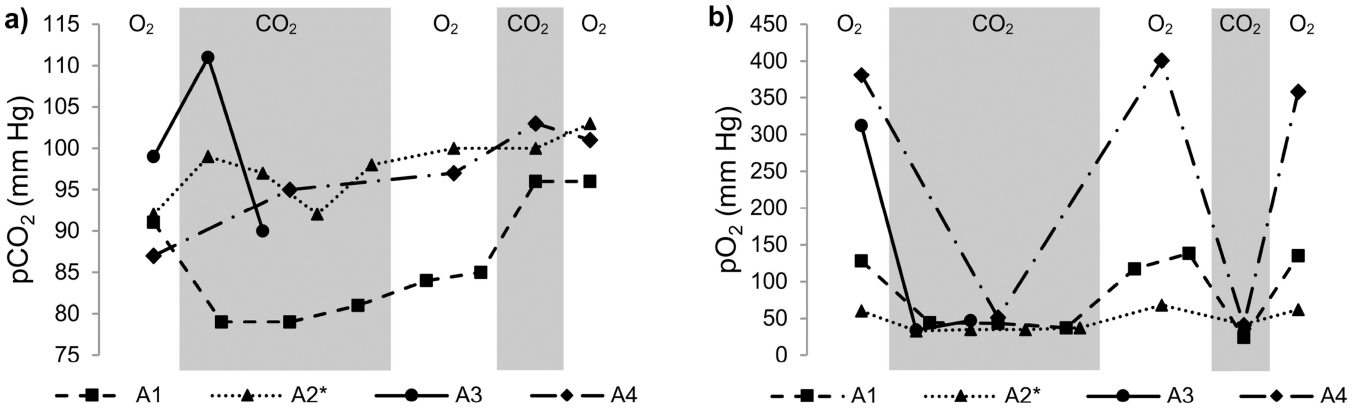

Fig. 4.

Plots showing the changes in the partial pressures of a) carbon dioxide (pCO_2) and b) oxygen (pO_2) in the blood upon exposure to oxygen or carbon dioxide. From the plot in a) no trend is evident in the partial pressure of carbon dioxide in blood, though in all pigs the partial pressure of carbon dioxide in the blood ends slightly higher than the baseline value. However, the plot in b) shows a decrease in the partial pressure of oxygen in the blood upon exposure to carbon dioxide and an increase upon exposure to oxygen, which in general tracks with the observed changes in the twinkle power.

*Blood samples from Pig 2 were venous rather than arterial samples.

Table 1

List of stable and varying parameters analyzed in the blood and urine.

	Stable Parameters	Varying Parameters
Blood:	None	**Blood:** Bicarbonate (mEq/L)
Urine:	Color	Base Excess (mEq/L)
	Appearance	pH
	Glucose	pCO_2 (mm Hg)
	Ketone	pO_2 (mm Hg)
	Bilirubin	O_2 saturation (%)
	Casts	**Urine:** Carbon Dioxide (mEq/L)
	Bacteria	pH
	Mucus	Specific Gravity
	White Blood Cells	Protein (mg/dL)
	Epithelials	Blood
	Crystals	Red Blood Cells